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Determination of Okadaic acid by Measuring 50% Acid Phosphatase Activity Inhibitory Concentration ($APIC^{50}$)

by

Koichi SHIRAKI*[1], Akira SUGITANI*[1], Makoto KAWAI*[1]

and Takeshi YASUMOTO*[2]

Foreword

The growth of tetrahymenae is singularly inhibited by diarrhetic shellfish toxins (DST). Its 50% growth inhibitory concentration (GIC_{50} [1]) corresponds to that of 1 mouse unit (MU)/20 mL medium. The DST-based 50% acid phosphatase activity inhibitory concentration ($APIC_{50}$) of tetrahymenae also shows good agreement. This is why we reported these facts for a practical DST determination method [2] based on GIC_{50} and $APIC_{50}$. The DST used in the present study was partially purified poison (mainly DTX3) extracted from poisoned scallops. The so far reported DST comprise several types of toxins, viz. okadaic acid (OA[3]) dinosphysistoxin 1.3 (DTX1, DTX3[3][4]), pectonotoxin 1-5 (PTX1-5[3]) and yessotoxin (YTX[5]).

We report here on our tests in using the GIC_{50}, $APIC_{50}$ and MU method to determine purified OA and anew separated PTX1, PTX2, YTX.

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Materials and testing method

1. Samples

OA Solution: We added OA to sterilized media and made preparations so as to have sample solutions adjusted to a poison amount of 1MU/mL.

PTX1 Solution: We added PTX1 to sterilized media and made preparations so as to have sample solutions adjusted to poison amounts of 2 MU/mL and 20 MU/mL.

PTX2 Solution: We proceeded as we did for the PTX 1 solution.

YTX: We proceeded as we did for the PTX1 solution.

2. Reagents

Medium: Same as in the separate report [2].

Acid phosphatase (acid phos) reagent: Same as in the separate report [2].

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3. Equipment

Centrifugal separator: Kubota KR 702

Other equipment: Same as in the separate report [2].

4. Measurement of poison amounts

We used the same mouse lethal tests as in the separate report [2].

5. Tetrahimenae and their culture method

We used the same method as in the separate report [2].

6. Tetrahimena growth inhibition tests.

We added according to the separate report [2] 100 mL each of the materials and of the sample prepared by method 1 to 100 mL Erlenmeyer flasks, and prepared (medium volume of 20 mL) a 0 - 1.0 MU concentrations series of the various toxins. We insulated 0.2 mL of the previous tetrahimena culture solution into the particular Erlenmeyer flasks (a concentration of 2.000 cells/mL of medium is obtained) and performed still culture at 28°C for 24 hours.

a) GIC_{50}

We added a few drops of 3.7% formalin solution to the above still culture solution so as to stop the ciliary movement, and counted the cells with a Buerger-Turck* type cytometric plate.

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*Translator's note: A guess; the original says: "Byurugerutiuruku".

b) $APIC_{50}$

We gathered the cultured cells by centrifugation (2,000 rpm, 5 min) washed twice with 30 mL of 0.5% brine and allowed to float in 10 mL of 0.5% brine. We destroyed the cells with an ultrasound crusher, added 0.1 mL of the supernatant to test tubes containing 0.5 mL substrate buffer solution (we used an acid phos measuring kit from Wako) and after heating for 30 min at 37°C in a thermostatic jar, we added 5 mL of 0.05 N sodium hydroxide solution for colour development. We then measured the acid phos activity at the 405 mm wavelength.

Results and discussion

1. Tetrahimena growth inhibition (GIC_{50})

We compared the tetrahimena growth in the concentration series of the various toxins (0.5 - 20 MU/20 mL media) with the controls (tetrahimena growth figures of media containing no toxins). The results are shown in Fig. 1.

The abscissa shows the poison amounts per 20 mL of medium. The lower-level figures show the MU-converted values per 1 g of sample, and 0.05 MU/g is the regular poisoning standard. The ordinate gives the relative growth rate in the media containing the toxins when the controls are taken as 100.

The relative growth rate with 1 MU of OA shows 50.6% (n=7) like for DTX3, and agreed well with the regular poisoning standard value of 0.05 MU/g. In the 0.5 - 20 MU range, PTX1, PTX2 show at 93.4 - 100.3 % (n=7) about the same values as the controls. There seemed to be no tetrahimena growth inhibition.

YTX shows 39.6% with 20 MU, and was seen as somewhat inhibiting the growth of tetrahimenae.

2. The acid phos activity of tetrahimenae ($APIC_{50}$)

We measured the intracellular acid phos activity of the tetrahimenae with the various poison amounts (0.5 - 20 MU/20 mL media) of the particular toxins, and compared in terms of relative activity rates when taking the acid phos activity of the controls (no toxins contained) as 100. The results are shown in Fig. 2. With 1 MU of OA, the acid phos activity inhibition rate is 48.1% (n=7) and this like DTX3 agreed well with the regular poisoning standard value of 0.05 MU/g.

Between 0.5 and 20 MU, PTX1, PTX2 show at 95.7 - 100.4 % about the same values as the controls. At 20 MU, YTX was 40.8 %.

Among these DST, the OA, DTX3 substances (the 7th position of OA is acylated and the 35th position is methylated) are polyethyl fatty acids, and the PTX1, PTX2 are polyethyl lactonized compounds.

YTX is a compound based on the fusion of 11 ether rings of the $C_{55}\ H_{80}\ O_{21}\ S_2\ Na_2$ molecular formula.

The existing studies and food poisoning cases have demonstrated that all these have mouse lethal characteristics and that only the polyether fatty acid system has also diarrhetic poison characteristics 3)6).

However, the relationship between these structures and the diarrhea-causing characteristics (food poisoning) has not been clarified.

The present tests have demonstrated that only this diarrhetic poison inhibits strongly the growth of tetrahymenae. These facts suggest that DTX1 which has a similar structure could display toxicity with regard to tetrahimenae. It will be interesting to study the poisoning mechanism of these polyethyl fatty acid systems with regard to tetrahimenae.

Summary

Using tetrahimenae, we measured GIC_{50}, $APIC_{50}$ on the following DST, viz. OA, PTX1, PTX2 and YTX.

1. In OA, the GIC_{50}, $APIC_{50}$ like DTX3 agreed with the 1 MU/20 mL (regular poisoning standard value of 0.05 MU/g).

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2. YTX showed a 40% inhibition rate in a 20 MU/20 mL medium and was slightly toxic with regard tetrahimenae.

3. PTX1, PTX2 were not seen to inhibit the tetrahimenae in 0.5 - 20 MU/20 mL media.

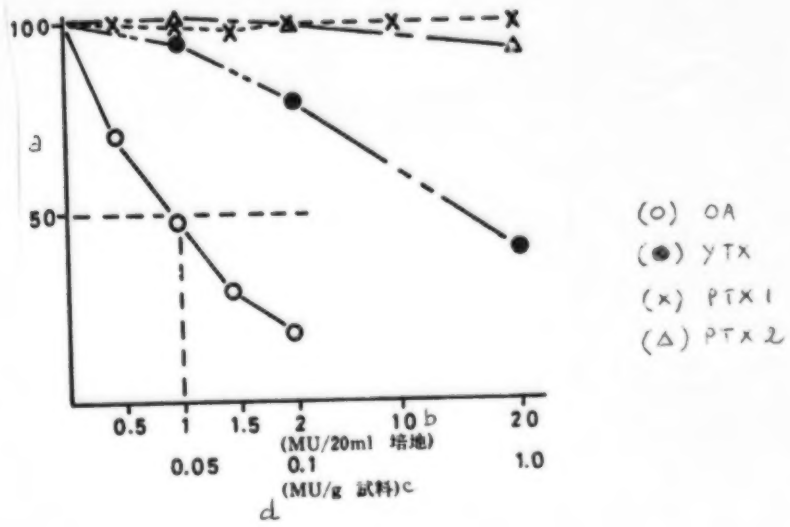
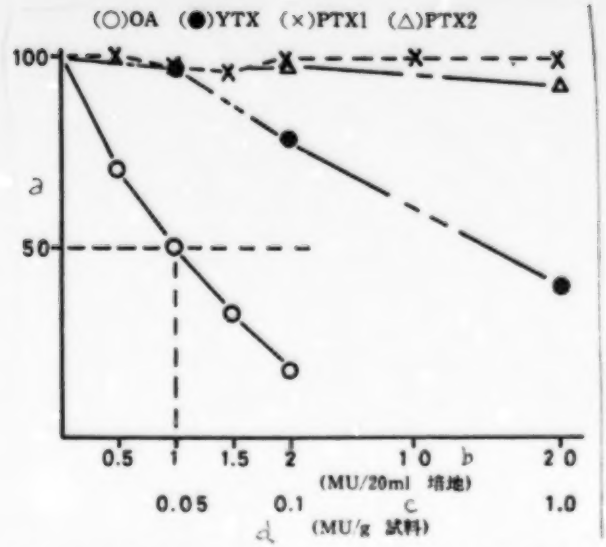
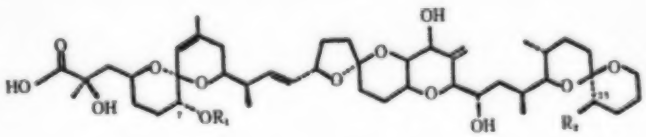


Fig. 1: Tetrahimena growth inhibition rate based on diarrhetic shellfish poison.
a. relative growth rate (% of control) b. medium, c. sample, d. amount of poison.



(o) OA
(●) YTX
(×) PTX 1
(△) PTX 2

Fig. 2: Acid phosphatase activity inhibition rate based on diarrhetic shellfish poison.
a acid phosphatase activity (% of control), b medium, c sample
d poison amount



a. オカダ酸：R_1＝H，R_2＝H
b. ディノフィシストキシン－1：R_1＝H，R_2＝CH_3
b. ディノフィシストキシン－3：R_1＝acyℓ，R_2＝CH_3

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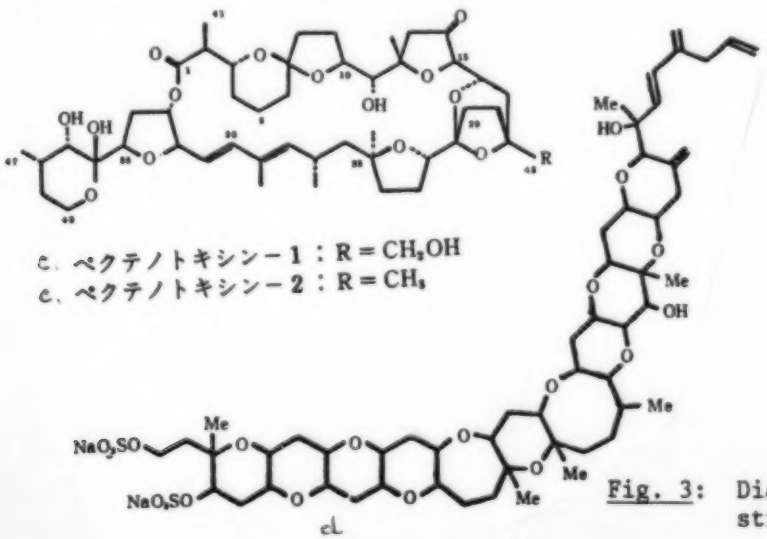

c. ペクテノトキシン－1：R＝CH_2OH
c. ペクテノトキシン－2：R＝CH_3

Fig. 3: Diarrhetic shellfish poison structure

a okadaic acid
b dinophysis toxin
c pectenotoxin
d yessotoxin

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